36. (Amended) The analyte binding array of claim 35, wherein the analyte binding partner forms a multi-layer matrix in the sorbent zone, the matrix extending up to 200 nm vertically from the surface of the substrate.

Please add the following new claims 37-42:

37. (New) The binding assay of claim 1 or 33, wherein the binding partner is immobilized on the surface of the substrate by covalent immobilization.

38. (New) The binding assay of claim 1 or 33, wherein the binding partner is immobilized on the surface of the substrate by non-covalent immobilization.

39. (New) The analyte binding array of claim 23 or 35, wherein the binding partner is immobilized on the surface of the substrate by covalent immobilization.

40. (New) The analyte binding array of claim 23 or 35, wherein the binding partner is immobilized on the surface of the substrate by non-covalent immobilization.

41. (New) The kit of claim 26, wherein the binding partner is immobilized on the surface of the substrate by covalent immobilization.

42. (New) The kit of claim 26, wherein the binding partner is immobilized on the surface of the substrate by non-covalent immobilization.

REMARKS:

Claims 1, 23, 26, 33, 35, and 36 are amended; marked up versions of the amended claims are attached hereto pursuant to 37 C.F.R. § 1.121(c)(ii). New claims 37-42 are added. The support for claims 37-42 can be found on page 17, lines 24-30. No new matter is introduced. Claims 1-42 are pending in the application.

irregular topology extending up to 200 nm vertically from the surface of the film [substrate]," wherein "each avidin molecule occupies 6nm^3 in the dry matrix."

Based on this description in the specification, a maximum number of molecular layers in the avidin matrix can be calculated. The shape of each avidin molecule can be approximated as a sphere. One can calculate a diameter of an avidin molecule based on a well-known formula: $V = \frac{1}{6} \times \pi \times d^3 \approx 0.524 \times d^3$ or $d = \sqrt[3]{\frac{V}{0.524}}$, wherein V is a sphere's volume and d is a sphere's diameter. Accordingly, the diameter of a 6nm^3 avidin molecule is $d = \sqrt[3]{\frac{6}{0.524}} \approx 2\,\text{nm}$. A sorbent zone extending up to 200 nm vertically from the surface of the substrate has up to $200 \div 2 = 100$ layers of avidin molecules. Therefore, the specification teaches that a maximum number of avidin layers is about 100.

The specification also teaches an average number of molecular layers in the avidin matrix. On line 19, page 19, the specification states that "the dry residue composing one 'spot' has a volume on the order of 6×10^{-11}cm^3," which equals 60 μm^3. Also, the specification indicates on lines 22-24 of page 19 that the structure of the spot is obtained by depositing 100 picoliters of avidin solution. Then, on lines 32-33 of page 19, the specification notes that "[i]f the printed volume is increased from 100 picoliters to 1 nanoliter, the spot diameter roughly doubles, to 200 microns." Thus, the diameter of the spot obtained by depositing 100 picoliters is about 100 μm.

Based on this description in the specification, an average number of molecular layers in the avidin matrix can be calculated. The shape of each sorbent zone or 'spot' can be approximated as a circle, the area of a circle $S = \frac{\pi \times d^2}{4} \approx 0.785 \times d^2$. Thus, the area of a 100 μm sorbent zone is about $0.785 \times d^2 \approx 8 \times 10^3\,\mu\text{m}^2$. Approximating the 3-D shape of the sorbent zones as a cylinder, one can calculate its height h as $h = \frac{V}{S} = \frac{60}{8 \times 10^3} \approx 7.5 \times 10^{-3}$ μm = 7.5 nm. Since the diameter of each avidin molecule is known (it is about 2 nm as calculated

above), one can calculate the number of layers in each sorbent zone 7.5 ÷2≈ 3 layers.

Therefore, contrary to the Examiner's belief, the present invention of a multi-layer matrix has been reduced to practice as demonstrated by Example 1 on pages 18-21. Example 1 provides data obtained by measuring immobilized sorbent zones of avidin molecules. Based on this data, any person having a basic understanding of chemistry and geometry can easily calculate the average and maximum number of monolayers contained within the matrix as demonstrated above. Thus, the specification provides a sufficient written description of the term "multi-layer matrix."[1]

Furthermore, the specification provides a sufficient written description of the term "multi-layer matrix," although the terminology used in the claim differs from that in the specification. In its recent decision in All Dental Prodx. LLC v. Advantage Dental Products. Inc. (64 U.S.P.Q.2d 1945, Fed.Cir.(N.Y.), Oct 25, 2002), the Federal Circuit observed that "the failure of the specification to specifically mention a limitation that later appears in the claims is not a fatal one when one skilled in the art would recognize upon reading the specification that the new language reflects what the specification shows has been invented." Additionally, all claims reciting the multi-layer matrix (claims 1, 23, 26, 33, and 36) have been amended to include the limitation "the matrix extending up to 200 nm vertically from the surface of the substrate" in order to place the upper limit on the size of the claimed multi-layer matrix.

Therefore, based on the description on page 19 of the specification and a general knowledge of molecular structure and geometry, those skilled in the art would have understood that applicants, at the time the application was filed, had possession of irregular multi-layer matrix of the analyte binding partner. Those

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[1] Please verify the accuracy of the calculations above. It appears that the estimate of 100 monolayers is high, particularly in view of your earlier statement of the matrix having about 3 monolayers. Is the statement of 100 monolayers being an upper limit on the size of the matrix and 3 monolayers being an average is correct?

skilled in the art would have understood that the multi-layer matrix extends up to 200 nm vertically and has as many as 100 monolayers and about 3 monolayers, on the average. Accordingly, applicants believe that the multi-layer matrix of the analyte binding partner was adequately described in the instant specification and does not introduce a new matter. Therefore, applicants submit that the rejections of claims 1-36 under 35 U.S.C. 112, first paragraph, should be withdrawn.

Claims 1-36 are rejected under 35 U.S.C. 112, first paragraph, as not enabled by the specification. The Examiner appears to believe that "neither the specification nor the claims teach how to define a multi-layer matrix ...[,] how to obtain such multi-layer matrices ...[,] what binding partners can or cannot be used in the complex being claimed[, and]... the specification does not include structural examples of a multi layer matrix" (§4 of the Office Action, page 4, first paragraph). Applicants disagree.

As discussed above, the present invention provides a specific example of a multi-layer matrix structure of avidin, including a summary of the Atomic Force Microscope (AFM) topology data. As shown above, the AFM data enables those skilled in the art to make structural calculations, including the determination of the average and maximum number of monolayers in the multi-layer matrix. Additionally, all claims reciting the multi-layer matrix (claims 1, 23, 26, 33, and 36) have been amended to include the limitation "the matrix extending up to 200 nm vertically from the surface of the substrate" in order to place the upper limit on the size of the claimed multi- layer matrix.

Furthermore, the present invention provides a general teaching on how to obtain such multi-layer matrices. The specification teaches the immobilization of relatively large quantities of the analyte binding partner (from about 10^5 to about 10^{12} molecules) on a relatively small area of a sorbent zone (from about 50 µl to about 500 µl) (page 4, line 31 – page 5, line 3). The specification, then, demonstrates in the Example 1, that by following this general teaching, one may obtain a multi-layer matrix of the binding partner. As explained on p. 8, lines 2-23, and as demonstrated in Figure 1 of the present invention, such a concentration of

the binding partner within the multi-layer matrix results in a 10-100 times higher analyte-binding capacity of the arrays of the present invention as compared to those of the prior art, and, thus, a 10-100 times higher sensitivity of the assay of the present invention as compared to the assay of the prior art.

Therefore, a reasonable amount of general guidance and a sufficient specific example are given by the specification with respect to the term "multi-layer matrix of binding partner." Accordingly, one skilled in the art would be able to practice the present invention without undue experimentation in light of the teachings of the instant specification. Consequently, applicants submit that claims 1-36 are enabled by the specification in their full scope and that the rejection under 35 U.S.C. § 112, first paragraph, should be withdrawn.

Claims 1-36 are rejected under 35 U.S.C. 112, first paragraph, as not enabled by the specification because, allegedly, the specification is only enabled with respect to binding assays in which the binding partner is immobilized covalently with a photoliable linker (§5 of the Office Action). Applicants respectfully traverse this rejection.

As discussed above, the present invention provides a general teaching of concentrating at least about 10^5 molecules and preferably as high as 10^{12} molecules of analyte binding partner within a sorbent zone having a diameter of about 100 μm to about 200 μm (page 11, lines 2-10). The immobilization may be covalent or non-covalent. For example, on page 17, lines 24-26, the specification describes conditions used for the covalent and non-covalent immobilization of the binding partner. For non-covalent immobilization, a 50 mM carbonate buffer at pH 8.2 was used for printing. For covalent immobilization, the buffer was 50 mM phosphate buffered saline at pH 7.4.

Therefore, a reasonable amount of general guidance and sufficient specific example are given by the specification with respect to both the covalent and non-covalent immobilization of the binding partner. Accordingly, one skilled in the art would be able to practice the present invention in its full scope without undue experimentation in light of the teachings of the instant specification.

Consequently, applicants submit that claims 1-36 are enabled by the specification in their full scope and that the rejection under 35 U.S.C. § 112, first paragraph, should be withdrawn.

Claims 1-36 are rejected under U.S.C. 112, second paragraph, as being indefinite (§ 7 of the Office Action). Applicants respectfully traverse this rejection.

The Examiner appears to believe that the term "multi-layer" is indefinite because it is not defined by the claim and the "specification does not provide a standard for ascertaining the requisite degree." Applicants disagree. As discussed above, the specification provides an upper limit on a number of monolayers of the immobilized analyte binding partner. Thus, the term "multi-layer matrix", read in view of the specification, is not indefinite. However, in order to expedite the prosecution of the present invention, applicants amended the specification by adding a limitation "the matrix extending up to 200 nm vertically from the surface of the substrate." This limitation explicitly recites the height limit on the multi-layer matrix, and, thus, provides an upper limit on the number of the monolayers in the matrix.

Applicants believe the foregoing amendments place the application in condition for allowance and early, favorable action is respectfully solicited.

If for any reason the Examiner finds the application other than in condition for allowance, the Examiner is requested to call the undersigned attorney at the Los Angeles telephone number (213) 337-6700 to discuss the steps necessary for placing the application in condition for allowance.

If there are any fees due in connection with the filing of this response, please charge the fees to our Deposit Account No. 50-1314.

Respectfully submitted,

HOGAN & HARTSON L.L.P.

Date: _____ By: _____

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Version with markings to show changes made:

IN THE CLAIMS:

Please replace the text of claims 1, 23, 26, 33, 35, and 36 with the following text:

1. (Amended Three Times) A binding assay for sensing analyte mass in a liquid sample, comprising:

a) immobilizing an array on a surface of a substrate, wherein the array comprises a plurality of microscopic sorbent zones, wherein a microscopic sorbent zone comprises a multi-layer matrix of an analyte binding partner, the matrix extending up to 200 nm vertically from the surface of the substrate;

b) contacting a defined volume of sample believed to contain an analyte with at least one microscopic sorbent zone, the analyte binding partner in the microscopic sorbent zone being present in excess relative to the analyte, so that any analyte present in the defined volume is substantially depleted from the sample and concentrated on the microscopic sorbent zone to form an analyte capture complex with the analyte binding partner;

c) tagging the analyte capture complex with a fluorescent label;

d) illuminating the microscopic sorbent zone with a laser in the absence of liquid; and

e) detecting fluorescence emissions from any microscopic sorbent zone having an analyte capture complex tagged with a fluorescent label, thereby determining the analyte mass harvested from the defined volume of sample.

23. (Amended Three Times) An analyte binding array for harvesting analyte from a liquid sample, the array comprising a plurality of microscopic sorbent zones immobilized on a surface of a substrate, wherein a microscopic sorbent zone comprises a multi-layer matrix of an analyte binding partner, the matrix extending up to 200 nm vertically from the surface of the substrate, the analyte binding partner being present in an amount sufficient to substantially

deplete the analyte from a sample and concentrate the analyte on the microscopic sorbent zone, the microscopic zone being from about 60 to about 500 μm in diameter and the sample containing about 10^5 to about 10^{10} molecules of analyte per 100 μl of the sample, wherein a volume of the sample is from 20 to 500 μl.

26. (Amended three Times) A kit for use in a binding assay that senses analyte mass in a liquid sample of a defined volume, comprising an analyte binding array and a container comprising labeled binding partner,

wherein the analyte binding array comprises a plurality of microscopic sorbent zones immobilized on a surface of a substrate, wherein a microscopic sorbent zone comprises a multi-layer matrix of an analyte binding partner, the matrix extending up to 200 nm vertically from the surface of the substrate, the analyte binding partner being present in excess relative to the analyte, so that any analyte present in the defined volume of the sample is substantially depleted from the sample and concentrated on the microscopic sorbent zone to form an analyte capture complex with the analyte binding partner, and

the labeled binding partner having a fluorescent label and being capable of binding to an analyte bound by an analyte binding partner.

33. (Amended) A binding assay for sensing analyte mass in a liquid sample, comprising:

a) immobilizing an array on a surface of a substrate, wherein the array comprises a plurality of microscopic sorbent zones, wherein each microscopic sorbent zone comprises a multi-layer matrix of an analyte binding partner, the matrix extending up to 200 nm vertically from the surface of the substrate, wherein the amount of the analyte binding partner immobilized in the sorbent zone with a diameter from 60 μm to 500 μm is from 10^9 to 10^{12} molecules;

b) contacting a defined volume of sample believed to contain an analyte with at least one microscopic sorbent zone, whereby analyte present in the defined volume is substantially depleted from the sample and concentrated on the

microscopic sorbent zone to form an analyte capture complex with the analyte binding partner;

c) tagging the analyte capture complex with a fluorescent label; and

d) detecting fluorescence emissions from the microscopic sorbent zone to determine the analyte mass harvested from the defined volume of sample.

35. (Amended) An analyte binding array for harvesting analyte from a liquid sample, the array comprising a plurality of microscopic sorbent zones immobilized on a surface of a substrate, wherein a microscopic sorbent zone comprises an analyte binding partner, the analyte binding partner being present in an amount from 10^9 to 10^{12} molecules per each sorbent zone with a diameter from 60 μm to 500 μm.

36. (Amended) The analyte binding array of claim 35, wherein the analyte binding partner forms a multi-layer matrix in the sorbent zone, the matrix extending up to 200 nm vertically from the surface of the substrate.